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NUTRITION REVIEWS

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Editor

FREDRICK J. STARE, PH.D., M.D., Associate Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 25 Shattuck St., Boston, Mass.

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NUTRITION REVIEWS

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NUTRITIONAL PROBLEMS IN THE FOOD INDUSTRIES

The importance of nutrition as a vital part of food processing and marketing has taken on new significance the last few years. There has been an ever increasing emphasis by the food industries in applying the findings of fundamental nutritional research to the betterment of the wide variety of processed food products.

Nutritionists and biochemists have been added to the research staffs of the food industries. They have developed comprehensive programs for research as well as for consultations with commercial and operating divisions. Bacteriologists, physiologists, zoologists, home economists, and medical research workers have joined with chemists and physicists to advance studies on the effects of processing, storage, marketing, and cookery, and also to counsel on educational advertising, food publicity, and industrial dietetics.

To supply more complete information for the best use of various foods, it is often necessary for the nutrition worker in industry to undertake basic studies of protein, fat, vitamin, mineral and carbohydrate metabolism, and requirements. Support for such researches in the universities and colleges is widely given by the food industry. This not only stimulates and assists fundamental scientific studies in these educational institutions, but establishes a closer contact with the university worker, increasing the effectiveness of his findings in their application for the public good.

Basically, there are two parts to a good nutritional program:

First, the foods must contain the necessary nutrients. As a dietary, they must contain all of the nutrients required and in adequate amounts.

Second, the people must be educated to eat foods in proportion necessary for good nutrition.

The food industry has a part in both phases, and processors have assumed the responsibilities associated with such a program.

A primary problem facing the food industry in the production of food with known and uniform nutrient content is that of securing improved raw products. The nutritional quality of these raw materials is a basic consideration in the production of high standard foods. Much research needs to be done in determining the diverse effects of different soils and climates on food quality. Research on plant and animal breeding is helping to contribute to the solution of this problem, and should be continued.

Raw material studies are essential for the determination of original vitamin and mineral content as a basis for the appraisal of processing losses. Assays are continued through the processes necessary to food preservation for surplus storage and wide distribution. Washing, trimming, and blanching in the prepackage preparation of foods are all causes of some nutrient loss. Canning in glass or tin involves relatively high temperatures to achieve satisfactory bactericidal action. One of the great problems of the food industry concerns the development of methods of eliminating bacterial deterioration without significant losses in nutrients. Electronic treatment or the use of bactericides which are harmless to man offer some promise.

The processing of foods as normally carried out by industry has little effect on either quantity or quality of protein. Fats and oils which are not separated from their natural food sources suffer little change in nutritive value except on long storage in

the fresh state. Processed fats and oils may lose much of their natural antioxidant content and need addition of protective agents. Diligent research is under way for such substances and on problems of flavor and odor instability.

A few years ago there was relatively little information in the literature concerning nutrient losses in foods during restaurant or home cookery. Most of the information about vitamins, minerals, and proteins which was tabulated for reference was based on the content of raw foods. Within the last few years, however, we have made some progress in developing methods for determining nutrient losses in processing and cooking. Nutritionists are becoming more aware of the importance of cooking and processing on the nutrients of foods. Food manufacturers are meeting the challenge by investigating and improving food processing from a nutritional standpoint. New and rapid methods of assay will continue to exert a great influence on the progress of nutrition research and ultimately on processing procedures.

Food industries, above all others, should be most interested in impressing the American public with the virtues of better nutritive practices. Major governmental effort has been concentrated on nutrition charts, the bread enrichment program, and industrial nutrition. Just how much these efforts have helped to give Americans a desire for better all-around dietary practices is difficult to evaluate.

Some publicity has been given to food consumption surveys made by various institutions and individuals, pointing particularly to vitamin shortages. Other than bread, the major food fortification has been the addition of vitamin A to margarine, first at 9000 units per pound, minimum, and now stepped up to 15,000. Iodized salt, and vitamin D-enriched milk represent additional accomplishments.

In 1941 the Food and Nutrition Board of the National Research Council provided a "yardstick of good nutrition." This board saw fit to compile and publicize a list of the names and quantities required daily of many of the essential nutrients on the basis of existing scientific evidence. It was intended that such data could be used in determining the nutritional adequacy of a dietary.

Taste and eye appeal will always play a major role in influencing food selection and these are based on early training of food habits. A major problem facing the establishment of a good nutritional regimen in America arises from faulty food habits. This can be overcome by sufficiently skillful and concerted education. Every food processor and every food agency can and should play its role in this educational program. Sound advertising as an educational device is often more effective than other channels. It should be truthful both by direct statement and by implication. Aggressive advertising is a constructive influence in bringing about improvements in food quality. It tends to create an incentive for improvement of a product.

A vastly increased advertising program on the importance of protein selection and the specific function of the various essential amino acids may well be expected.

Many industrial research programs are designed to determine the best use of individual products in dietary regimens. This is a forward step on the part of processors. This thought could well be extended to their educational work, by supporting the theme of a widely varied and thoughtfully selected dietary rather than concentrating on the questionable project of achieving single perfect foods or to proclaim nutritional superiority of a particular product over other good foods. When this principle is fully recognized there will be more education on the supplementary effect of each food item on the dietary as a whole, and less implication that one food item is better than another.

While many questions are yet unanswered with respect to the supplementary or sparing

action of one nutrient and food to another and the special requirements for many normal and abnormal conditions, it seems clear that our present knowledge is far beyond its application to the dietary of most of our people.

Such a condition merits our sober consideration. It calls for a program of education more effective than has heretofore been applied. No criticism should be made of the efforts previously carried on through the usual educational channels, unless it be to point out that these efforts have fallen far short of what is required.

There have been posters and bulletins by the millions. There have been lecture courses by the thousands. Community and government sponsored committees all across the country have been active and certainly all of these agencies have had beneficial effects in the educational program. Despite all this fine work it is believed by some that the greatest influence in making people nutrition conscious has come through the medium of paid advertising by the food industry. Here, then, is an important avenue for general education of the public in matters of nutrition and public health. Advertising specialists know how to get attention and how to influence public action. These advertising people must, however, become better educated in the field of nutrition.

The Nutrition Foundation has been helpful by supplying reliable information to teachers, science writers, newspaper editors, dietitians, home economists, public health officers, dentists, and medical practitioners. This is a worthy program because it informs those who are in a position to pass the information on to the public, but it needs to be supplemented and strengthened.

From these observations it is evident that the basic job of the food industries is to supply the American people with palatable, sanitary foods from which they can get a complete supply of all the required nutrients. Business succeeds only to the extent that it performs its function in serving society. While advertising and other forms of education are helpful and necessary, they cannot assure permanent success unless this basic function is fulfilled.

R. C. Newton[1],
H. E. Robinson[2],
Chicago, Ill.

[1] Member Scientific Advisory Committee, Representative from Industry;
[2] Member Food Industries Advisory Committee, Nutrition Foundation, Inc.

PRESENT KNOWLEDGE OF THE MINERALS IN NUTRITION (PART I)

Although the minerals constitute only a small portion of the body, like the vitamins, they enter into many vital physiologic functions. Unfortunately in the past decade, fundamental mineral metabolism studies have received less attention than they merit. Part of the neglect has been due to the difficulty in devising methods for studying the intimate relations of the minerals to physiologic functions, the difficulties in detecting borderline deficiencies, and also to the less dramatic effects of mineral deficiencies in human populations. The use of isotopes of various types may be the route through which mineral metabolism can be most effectively investigated in the future.

The animal body requires seven principal mineral elements, calcium, magnesium, sodium, potassium, phosphorus, sulfur, and chlorine, and at least six other minerals, the so-called "trace" elements, iron, copper, iodine, manganese, cobalt, and zinc. It is unknown whether fluorine, aluminum, and boron are nutritional essentials for animals or man.

Calcium is present in the body in larger amounts than any other positive mineral element; about 99 per cent being involved

in the construction and function of the bones and teeth. The relations of calcium to phosphate, magnesium, acid excretion, hormones, and vitamins present very complex problems. The small fraction of calcium not located in the skeleton exists partly as calcium ions and is of fundamental physiologic importance in the activity of nerves, muscles, and the heart, in the clotting of blood, and in the maintenance of membrane permeability.

As indicated by various dietary surveys (*Nutrition Reviews* **3,** *76, 106* (*1945*)), calcium is the mineral most likely to be deficient in the human dietary, yet it is impossible to diagnose early calcium deficiency clinically, hence clinical studies do not suggest that calcium deficiency is widespread. Chemical balance studies are useful, but these are affected materially by endocrine and various dietary factors, and by the length of time over which they are run (*Ibid.* **2,** *118* (*1944*)).

The ability to utilize the calcium in ingested foods varies tremendously among individuals. At least part of this variation is due to the composition of the rest of the diet. Calcium balance studies with human subjects on low and high protein intakes indicate that a high protein diet resulted in an absorption of 15 per cent of the dietary calcium in contrast to 5 per cent absorption by the subjects on a low protein diet (*Nutrition Reviews* **1,** *235* (*1943*)). Phytic acid has been shown to interfere with the absorption of calcium either by precipitation or by conversion into a form which is not absorbed from the intestine (*Ibid.* **3,** *61* (*1945*)). When phytase is present the salts of phytic acid can be hydrolyzed by enzymatic action. In the rat, calcium utilization is greatly reduced by the ingestion of oxalates or foods containing oxalates. If the diet contains a large amount of calcium, this reduced calcium utilization does not result in evidences of calcium deficiency (*Ibid.* **3,** *324* (*1945*)). The ingestion of large amounts of a low cost breakfast cocoa by rats resulted in a reduced calcium retention (*Ibid.* **2,** *68* (*1944*)). However, two medium priced cocoas did not produce any greater reduction in the calcium retention of rats than could be explained by their oxalate contents or about one-third the reduction in calcium retention produced by the same amount of the low cost cocoa (H. H. Mitchell and T. S. Hamilton, *J. Nutrition* **31,** *377* (*1946*)).

The Recommended Dietary Allowances adopted by the Food and Nutrition Board of the National Research Council in 1945 for calcium were: 0.8 g. daily for men and women, from 1.0 to 1.4 g. daily for children, and 1.5 and 2.0 g. daily during the latter half of pregnancy and in lactation (*Nutrition Reviews* **3,** *287* (*1945*)). Actually the most satisfactory intake of calcium at any stage in the development of man is difficult to assess accurately because of the many variables which affect its utilization, and the individual's ability to absorb what is ingested. Under these circumstances, it seems advisable that attempts be made to provide amounts as nearly those recommended as possible, along with a diet which is otherwise optimal in order to assure sufficient calcium utilization for normal physiologic well-being.

Phosphorus: About 80 per cent of the phosphorus in the body is in combination with calcium in the bones and teeth. Half of the remaining phosphorus is present in combination with proteins, lipids, carbohydrates, and other organic compounds in the muscles. The rest is distributed widely throughout the body in organic combination. Phosphorus compounds are essential in fat and carbohydrate metabolism and play a complex role in the chain of events occurring in muscle contraction and the transfer of energy.

T. B. Osborne and L. B. Mendel (*J. Biol. Chem.* **34,** *131* (*1918*)) showed that moderate restriction of the phosphorus intake in the growing rat resulted in a definite reduction in growth. The calcium-phos-

phorus ratio in the diet has a definite relation to the absorption and excretion of these elements. When either calcium or phosphorus is fed in excess, there is an increased excretion of the other element. Not only are liberal amounts of calcium and phosphorus in an optimum calcium-phosphorus ratio of roughly 1:1 necessary, but these must be accompanied by adequate vitamin D if skeletal tissue is to be formed and maintained normally. Inadequate or unbalanced diets with respect to calcium, phosphorus, and vitamin D result in rickets in infancy or childhood and osteoporosis in the adult, especially during pregnancy and lactation.

"Resistant rickets," where there is no associated renal disease, is a condition which is little understood. Studies with radioactive phosphorus given either in the postabsorptive state or with the regular food indicated that phosphorus could be readily absorbed in this disease. This is contrary to the previous concept of the malfunction occurring in resistant rickets and has led to the supposition that the failure lies in the calcifying mechanisms rather than in absorption (*Nutrition Reviews* **2,** *218* (*1944*)).

With regard to phosphorus allowances the Food and Nutrition Board stated:

"Available evidence indicates that the phosphorus allowances should be at least equal to those for calcium in the diets of children and of women during the latter part of pregnancy and during lactation. In the case of other adults the phosphorus allowance should be approximately 1.5 times those for calcium. In general it is safe to assume that if the calcium and protein needs are met through common foods, the phosphorus requirement also will be covered, because the common foods richest in calcium and protein are also the best sources of phosphorus" (*Nutrition Reviews* **3,** *287* (*1945*)).

Magnesium: Three fourths of the magnesium in the body is in combination with calcium and phosphorus in the complex bone salts, while the remainder is present in the soft tissues and body fluids. Actually, little is known of the physiologic roles of magnesium but an important function is as a catalyst for numerous enzymatic reactions. Under conditions of stress, magnesium is able to replace calcium in the bone salts to a small extent, yet in excess magnesium inhibits the calcification process.

H. D. Kruse, E. R. Orent, and E. V. McCollum (*J. Biol. Chem.* **96,** *519* (*1932*); *Ibid.* **100,** *603* (*1933*)) have produced a magnesium deficiency by feeding young rats a diet containing only 1.8 parts per million of magnesium. Within a few days the exposed areas of the skin in these animals became red due to vasodilatation and hyperemia; hyperirritability was observed, followed by cardiac arrhythmia, spasticity, and tetany.

One case of tetany in a child has been reported which was probably due to a magnesium deficiency and which was relieved by the oral administration of 0.3 g. of magnesium sulfate three times daily, with an accompanying increase in the plasma magnesium from 0.6 to 2.6 mg. per 100 ml. (*Nutrition Reviews* **2,** *189* (*1944*)). Several interesting associations between the parathyroid glands, dysfunction of the thyroids, renal insufficiency, and magnesium have been described (*Ibid.* **2,** *287* (*1944*)). No seasonal variation in the absorption of magnesium was observed to occur, unlike with calcium (*Ibid.* **2,** *153* (*1944*)). Little is known of the human requirement for magnesium.

Sodium: Sodium forms the largest fraction of the total base in the blood plasma and the extracellular fluids. As it occurs in the body, sodium is associated largely with the chloride and bicarbonate ions and functions with them to maintain the osmotic pressure and the ionic equilibrium of the body fluids and tissues. The amount of sodium within the human red cell is so small that its presence is questioned, although it is present in considerable quantities within the red blood cells of other species.

The use of sodium salts for the treatment of shock produced experimentally in animals

by scalding or by trauma, and the successful clinical trials of sodium lactate in the treatment of burn shock have been described (*Nutrition Reviews* **3,** *74* (*1945*)). It is interesting to note that potassium chloride was either ineffective or definitely deleterious in the treatment of shock in experimental animals, while isotonic solutions of sodium chloride appeared to be superior to hypertonic solutions. Oral administration of sodium chloride was as effective as injection. The actual human requirement for sodium is comparatively small. The normal amount of salt consumed daily per capita in the United States is around 10 g. or about 4 g. of sodium.

Potassium: Potassium is the principal cation within the cells where it functions in conjunction with phosphate, chloride, and bicarbonate. The potassium content of the red blood corpuscles is over thirty times that of the blood serum. Similar ratios exist between the potassium concentration within the tissue cells and that in the interstitial fluids. When new protoplasmic tissue is being produced, potassium is mobilized within the developing cells in sufficient quantities to meet their intracellular needs. There seems little doubt that some of the intracellular potassium must be bound so that it is not freely ionized and does not exert osmotic pressure aside from the whole molecule of which it is a part.

When rats were maintained on a diet low in potassium (0.1 per cent) but otherwise adequate, necrosis of the myocardial fibers and renal tubular epithelium was produced. Addition of potassium to the diet prevented the appearance of these changes (*Nutrition Reviews* **1,** *10* (*1942*)). More recently potassium deficiency in dogs has been described by W. R. Ruegamer, C. A. Elvehjem, and E. B. Hart (*Proc. Soc. Exp. Biol. Med.* **61,** *234* (*1946*)). When placed on a potassium deficiency ration the dogs failed to grow and developed a paralysis. Both conditions responded favorably to treatment with potassium. The requirement of man for potassium is unknown but is apparently easily met since almost all natural foods are good sources of potassium.

Chlorine: Chlorine composes about 0.1 per cent of the adult body; the chlorides of the blood compose about two thirds of the blood anions. The cerebrospinal fluid contains the highest chloride concentration, followed by the interstitial fluids, the lymph, serous transudates, exudates, and the gastrointestinal secretions.

Human diets usually contain adequate amounts of chlorine, except during periods of excessive loss through diarrhea, sweating, and certain endocrine and metabolic disturbances when additional amounts may be required.

Sulfur: All cells of the body contain sulfur, located principally in the sulfur containing acids of the proteins: cystine, cysteine, methionine, and ergothionine. Only small amounts of inorganic sulfur occur in the blood and tissues. Sulfur also occurs united to the carbohydrates, for example as chondroitin sulfuric acid which is located in the cartilage, tendon, and the organic matrix of the bone. Sulfur is also a constituent of the sulfatides, glutathione, insulin, biotin, and thiamine.

A large part of the sulfur utilized by the body occurs in cystine and methionine. W. C. Rose and co-workers (*J. Biol. Chem.* **114,** *lxxxv* (*1936*)) showed that methionine was an essential amino acid and could not be replaced by cystine.

The sulfur balance of the domestic fowl has been determined (*Nutrition Reviews* **3,** *279* (*1945*)). During rest periods, a retention of 44 mg. of sulfur daily was observed. When molting the hens lost around 25 mg. of sulfur per day in the feathers shed, with the result that there was an over-all loss of about 0.6 mg. of sulfur daily. In periods of low egg production, the hens were storing sulfur; but when a production of 50 per cent or higher was reached there was a substantial loss of sulfur daily.

FOLIC ACID

On May 29, 1946 the New York Academy of Sciences sponsored a conference on folic acid at the American Museum of Natural History in New York City. This was made the occasion for announcing the chemical structures and describing the synthesis of folic acid and certain derivatives. Also included in this symposium were papers on the pharmacology of synthetic folic acid, the production and treatment of folic acid deficiency in experimental animals, and a summary of the use of this vitamin in the successful therapy of sprue, pernicious anemia, and other macrocytic anemias.

Several reviews in this journal (*Nutrition Reviews* **3**, *165 (1945)*; **4**, *11, 13, 15, 102 (1946)*) have summarized the researches of many investigators in various fields, which have led to the conclusion that there was widely distributed in natural products (liver, yeast, spinach, etc.) a group of interrelated substances which were variously referred to as vitamin M, folic acid, Lactobacillus casei factor, and SLR factor, depending upon the source of the material and the species of organism used for test purposes (i.e. chicks, monkeys, rats, fish, and L. casei). The various forms of this dietary factor differed in their activity toward different test objects in their ability to eradicate the signs of the specific deficiency (anemia, failure to grow, etc.). There was much evidence that the several factors all had a common chemical structure but differed from each other by (a) the presence of extra chemical groups attached to the parent molecule which made these forms available only to certain organisms, or (b) the absence of certain parts of the common structure which restricted availability to those organisms which could synthesize the missing moiety.

Observations now made possible with the new synthetic compound have in general confirmed the above predictions. But of greater importance is the fact that the ready availability of pure substances and the previous knowledge from animal studies that folic acid was in some way related to hematopoietic activity has led to the successful therapeutic trials of folic acid (L. casei factor) in many macrocytic anemias, principally in sprue and pernicious anemia. It would be difficult to overestimate the scientific significance of these observations. They provide a new and valuable therapeutic agent for two serious and troublesome diseases, and open the way for studies on the chemical pathology and etiology of pernicious anemia, sprue, and related diseases. They also afford a new basis for practical applications of the science of nutrition in the protection of public health.

Details of the methods used for the isolation of two compounds from natural sources, which show folic acid activity, were described: namely, "Liver L. Casei Factor" and "Fermentation L. Casei Factor." The latter compound was isolated from the filtrate obtained from an anaerobic fermentation of a diphtheroid-type organism. Details of the methods used for establishing the two chemical structures, as well as procedures for the syntheses were also described. Many of these details have recently been published (*Science* **103**, *667 (1946)*).

The "Liver L. Casei Factor" (active for chick, rat, monkey, turkey, guinea pig, L. casei, and S. lactis R (faecalis)) proved to be composed of three main chemical groupings which upon hydrolysis appeared as: (1) a two-ringed nitrogen compound, a pteridine, (2-amino-4-hydroxypteridine-6-carboxylic acid); (2) para-aminobenzoic acid; and (3) glutamic acid. These are linked through the six position of the pteridine and the amino group of para-aminobenzoic acid, which in turn is linked through its carboxyl group to the amino group of glutamic acid. The name pteroyl glutamic acid was proposed for the complete compound. In addition to the above evidence

for the structure, additional information was obtained by synthesis.

The chemical structure of the "Fermentation L. Casei Factor" differed from the "Liver L. Casei Factor" in containing two additional glutamic acid groups. The "liver factor" was obtained as one of the products of the hydrolysis of this more complex molecule.

Synthesis was obtained (15 per cent yield) by simultaneous reaction of appropriate derivatives of the three units, or by a stepwise process with subsequent purification. When para-aminobenzoic acid instead of p-aminobenzoyl-1(+)-glutamic acid was allowed to react with the pteridine, a compound was obtained which was active for S. lactis R (SLR) but not for L. casei and the chick.

Para-aminobenzoic acid (PAB) is known to most readers because of its inhibitory effect on "sulfa" drug action and its chemotherapeutic value in rickettsial diseases (*Nutrition Reviews* **4,** *87 (1946)*). Glutamic acid will be recognized as one of the amino acids. It is of interest that a pteridine was first isolated as one of the pigments in the wings of butterflies.

Pharmacologic studies show that pteroyl glutamic acid is essentially nontoxic. The lethal dose is reported as 125 to 600 mg. per kilogram, depending upon the species of test animal. This is five hundred to five thousand times the therapeutic doses which have been used successfully in sprue and pernicious anemia.

While pteroyl glutamic acid has led to favorable response in a number of cases of pernicious anemia, it seems certain that liver extracts contain active material other than this compound since the potency of such extracts is greater than can be accounted for by their pteroyl glutamic acid content.

THE EXCRETION OF WATER SOLUBLE VITAMINS BY THE SKIN IN TROPICAL CLIMATES

The excretion of vitamins by the skin has attracted attention for some years now (*Nutrition Reviews* **1,** *355 (1943)*; **2,** *219 (1944)*; **3,** *5 (1945)*), and has recently received further study by the Animal Nutrition group at the University of Illinois, who have measured the excretion by the skin of materials and vitamins under various experimental conditions. H. Spector, T. S. Hamilton, and H. H. Mitchell (*J. Biol. Chem.* **161,** *145 (1945)*) have reported observations on the loss of pantothenic acid from the human body. They point out that if it is established that in tropical environments increased losses of pantothenic acid in the sweat represent more than a diversion of excretion from the kidneys to the sweat glands, then the finding may have some practical significance in human nutrition. They are careful to emphasize that this will be true only if the essentiality of pantothenic acid in human nutrition is firmly established; and further to emphasize that the demonstration of a therapeutic value of a substance does not necessarily imply that a dietary deficiency has been corrected by it.

The general experimental conditions were the same as in their experiments on iodine excretion (*Nutrition Reviews* **4,** *169 (1946)*). Pantothenic acid was determined microbiologically in dermal excretions and urine, using Lactobacillus arabinosus as test organism, grown on the basal medium of W. A. Krehl, F. M. Strong, and C. A. Elvehjem (*Ind. Eng. Chem. (Anal. Ed.)* **15,** *471 (1943)*). Five young men were the subjects; the dermal secretions were collected for eight hour periods and the urines for twenty-four hour periods.

The undiluted sweat of the subjects on the unsupplemented diet contained on the average 3.8 micrograms of pantothenic acid

per 100 ml. and there was no increase after daily supplementation with 16 mg. of calcium pantothenate plus the pantothenic acid present in 12 mg. of brewers' yeast (estimated at 2 mg.) for one week. With an unsupplemented diet the mean dermal excretion of pantothenic acid under comfortable conditions was 5.1 micrograms per hour, while under hot moist conditions which increased the net loss of body weight by 176 per cent it rose to 27.7 micrograms per hour. An increase in the urinary excretion of pantothenic acid also occurred on changing from comfortable to hot moist conditions, although the urine volume was reduced to one-half or one third of the initial value. Under the hot moist conditions, almost one third of an 18 mg. dose of pantothenic acid was recovered in the urine. The data suggest that a small increase in total loss of pantothenic acid in urine and sweat may occur under conditions of profuse sweating induced by environmental conditions.

B. C. Johnson, H. H. Mitchell, and T. S. Hamilton (*J. Biol. Chem.* **161,** *357* (*1945*)) have reported similar studies on para-aminobenzoic acid and inositol. Four young men were the experimental subjects; in measuring inositol a modification of the method of R. J. Williams, A. K. Stout, H. K. Mitchell, and J. R. McMahan (*Univ. Texas Pub.* **4137,** *27* (*1941*)) was used with Saccharomyces cerivisiae as the test organism, and for para-aminobenzoic acid the method of M. Landy and F. Streightoff (*Proc. Soc. Exp. Biol. Med.* **52,** *127* (*1943*)) was used. The concentration of inositol in sweat was 21 micrograms per 100 ml. (mean for the 4 subjects) and no significant increase in this level occurred after dosing the subjects with 50 mg. of inositol daily for three days. Much less para-aminobenzoic acid was found in the sweat; the mean value was approximately 0.3 microgram per 100 ml. and again it was not significantly raised by dosing the subjects with 100 mg. or 200 mg. of para-aminobenzoic acid daily. Under comfortable conditions the mean hourly loss of inositol in sweat during an eight hour "exposure" period was 0.027 mg. and the urinary hourly loss in twenty-four hours was 0.62 mg. Under hot moist conditions, the corresponding sweat loss was 0.118 mg. and the urinary loss 0.494 mg. per hour.

The excretion of ascorbic acid and dehydroascorbic acid has been reexamined by J. B. Shields, B. C. Johnson, T. S. Hamilton, and H. H. Mitchell (*J. Biol. Chem.* **161,** *351* (*1945*)). These authors again showed that rubber contains water extractable material which titrates as ascorbic acid with the indophenol dye, so that false high results may be obtained by this method, if rubber is used for collecting the sweat. They therefore adopted the method of J. H. Roe and C. A. Kuether (*Ibid.* **147,** *399* (*1943*)) for measuring both ascorbic acid and its oxidized form. Very small concentrations of each form were found in the sweat; in the 4 subjects the mean ascorbic acid was 19 micrograms per 100 ml. and the dehydroascorbic acid 70 micrograms per 100 ml. After dosage with ascorbic acid the dehydroascorbic acid content of sweat almost doubled, but the ascorbic acid content decreased.

These results serve to support the view (*Nutrition Reviews* **1,** *355* (*1943*)) that "There does not appear to be convincing evidence that the vitamin losses in sweat are large compared to the daily excretion in the urine."

FORMATION OF THYROXINE IN IODINATED CASEIN

It was shown by W. Ludwig and P. von Mutzenbecher (*Z. f. physiol. Chem.* **258,** *195* (*1939*)) and independently by C. R. Harington and R. V. P. Rivers (*Nature* **144,** *205* (*1939*)) that crystalline dl-thyroxine can be isolated from the products of alkaline hy-

drolysis of iodinated casein. This finding was confirmed by E. P. Reineke and C. W. Turner (*J. Biol. Chem.* **149,** *555 (1943)*), who also reported (*Ibid.* **149,** *563 (1943)*) that the natural l isomer can be isolated after hydrolysis with acid. The last named investigators have now reported further details of the effect of various factors on the formation of the thyroxine in iodinated casein. With other collaborators, they first investigated the methods for the quantitative determination of thyroxine in iodinated casein having thyroidal activity (E. P. Reineke *et al. Ibid.* **161,** *599 (1945)*). Because there was a question concerning the specificity of the chemical methods previously available for the determination of thyroxine in thyroid tissue it had been necessary in earlier work to depend on biologic methods for quantitative estimates of the potency of the iodinated casein preparations. A modification of the method devised by N. F. Blau (*Ibid.* **102,** *269 (1933)*, **110,** *351 (1935)*) for use with thyroid was worked out. The method presented is based on the hydrolysis of the iodinated casein with 40 per cent barium hydroxide, extraction of the thyroxine with n-butanol, purification of the butanol extract by washing with 4 N sodium hydroxide solution containing 5 per cent sodium bicarbonate, evaporation of the butanol, and determination of the iodine content of the residue.

It was demonstrated that thyroxine could be recovered quantitatively from known solutions by the extraction procedure, and that hydrolysis of thyroxine with 40 per cent barium hydroxide for twenty hours (the time for hydrolysis in the method given) caused a destruction of only 6 to 7 per cent of the thyroxine taken. Di-iodotyrosine had a negligible effect on the determination. For a number of preparations of iodinated casein made under different conditions comparisons of the results obtained by the chemical method with those obtained in bioassays were made. The criterion of response in the bioassays was the metabolic stimulation produced by intraperitoneal injection of the preparations into guinea pigs. Good agreement was obtained between the two methods, from which it was concluded that nonthyroxine iodine compounds which accompany thyroxine in the chemical procedure either contribute only a small fraction of the total iodine measured, or else produce a metabolic response in guinea pigs comparable to that produced by thyroxine.

On applying the new chemical procedure to a study of the effect of conditions on the formation of thyroxine in iodinated casein, E. P. Reineke and C. W. Turner (*J. Biol. Chem.* **161,** *613 (1945)*) showed that, following the iodination of the casein, the yield of thyroxine is markedly increased by raising the incubation temperature to 70° and agitating the solution vigorously. Under conditions described in earlier reports of the authors, it had been shown that the amount of iodine added to the casein was critical, and that maximum thyroxine formation was obtained by the addition of 4.5 to 5 atoms of iodine per molecule of tyrosine in the casein. Under the conditions now employed the thyroxine yield increases until 7 atoms of iodine have been added.

The addition of metallic compounds was also studied, because earlier work had indicated that the reaction was catalyzed by a brass stirrer used to make some preparations. In studying this effect it was found that the most powerful catalysts were manganese compounds, particularly the oxide Mn_3O_4 and the oxides produced by the reduction of $KMnO_4$ by glucose. The fact that increased thyroxine formation occurs if the casein is iodinated at 38 to 40° and then incubated with vigorous agitation in air at 70°, whereas incubation under nitrogen decreases the yield, leads the authors to favor the hypothesis that during the iodination di-iodotyrosine is formed and during the subsequent incubation two

molecules of di-iodotyrosine couple oxidatively with the elimination of one side chain to form thyroxine.

The catalytic effect of manganese on the thyroxine is of interest in view of the report of T. W. Ray and L. J. Deysach (*Proc. Soc. Exp. Biol. Med.* **51**, *228* (*1942*)) that the thyroid gland has a particular ability to store manganese, and also that the injection of manganese causes an increase in the oxygen consumption of guinea pigs. The combination of this information with the new facts now reported has led the authors to postulate that manganese may act catalytically *in vivo* in promoting the formation of thyroxine in the thyroid gland, as well as *in vitro* in promoting the formation of thyroxine in iodinated casein.

NUTRITION AND SUSCEPTIBILITY OF MICE TO PNEUMOCOCCAL INFECTION

The relation between diet and the susceptibility of man and animals to various infections has been a subject of considerable interest for many years. It is well known from experiences in time of famine and from observations of the frequency of spontaneous and induced infections among laboratory animals that, in general, inadequate diets lead to lowered natural resistance. However, this is not necessarily the case, as has been shown by recent studies in which host resistance was actually increased by dietary deficiencies (*Nutrition Reviews* **2**, *178* (*1944*)). It is not surprising that there is a lack of uniformity in host response to the combined insults of infectious agents and dietary restrictions. All infectious processes are not the same. Each may show specific characteristics. Likewise, each of the various nutritional deficiencies affects the body in a different way. The relation of diet and nutrition to infection is not one problem but many, and much more careful study will be required before the subject is well understood.

A recent paper by G. H. Hitchings and E. A. Falco (*Proc. Soc. Exp. Biol. Med.* **61**, *54* (*1946*)) points out the necessity of considering the direct effects of the host diet on the invading infectious agent, in addition to its effect on the efficiency of the host mechanisms which are involved in combating the invasion.

Albino mice maintained on commercial mouse feeds were found to be much more susceptible to fatal infection with type I pneumococcus than were mice receiving a standard purified basal diet to which had been added the known essential vitamins. For example, only 11 per cent of 56 mice (18 to 24 g.) receiving fox chow survived an intraperitoneal injection of a standard inoculum, while 86 per cent of 52 mice receiving the basal laboratory diet survived.

The inoculum was "0.5 ml. of a dilution of a 17 hour culture representing 1.0 ml. of a 10^{-6} concentration. The actual number of organisms injected varied from 235 to 700 as determined by plate count. The pneumococcus, Type I, SV-1 strain, was grown on proteose-peptone-blood broth, and was passed through mice at weekly or biweekly intervals."

The resistance to pneumococcal infection of the mice on the basal diet was so great that it was not until the concentration of the culture reached 10^{-3}, the equivalent of about 100,000 minimum lethal doses for the mice on the commercial chow (controls), that any considerable proportion (54 per cent) succumbed to the infection. The same experiment was repeated on a number of occasions with adequate controls so that there seems to be no doubt about the reproducibility of the observations. It would appear, however, from the data presented that the strain of mice used even when on chow diets was not as susceptible to pneumococcal

infection as those generally used for this type of work. It should be added, however, that this does not explain the great differences in susceptibility which were observed as a result of the different diets.

Another interesting point is the short time of treatment with the basal diet required to effect this change in susceptibility. If the mice were changed from the chow diet to the basal diet at the time of injection, some increased resistance to the infection was evident; indeed, even a single day on the basal diet definitely increased the resistance.

The authors point out that their results can be explained by the presence of some protective factor in the purified diet which is missing from mixed diets, or the presence in the mixed diet of a factor which increases the susceptibility of mice to pneumococcal infection. Although the first possibility has not been excluded, they rather favor the latter possibility as best fitting their results to date. On this hypothesis some factor essential and conducive to the rapid multiplication of the pneumococcus in the body must be present in the mixed diet but absent or in low concentration in the synthetic diet. This hypothetical substance would not be necessary or at least so essential for the nutrition of the mouse as for the pneumococcus. It would, in fact, be present in minimal concentration in the blood and body fluids of the mouse, except in so far as higher levels were brought about by a continuous supply from exogenous sources. This would explain the rapid change in susceptibility of the mouse which occurs when the dietary regimen is changed.

It has been shown that none of the known dietary essentials influences this decreased susceptibility to infection. The final proof of the authors' hypothesis awaits the preparation of active concentrates or pure products from the food mixture leading to the effect.

The idea that some foods may contain substances favoring the multiplication of certain infectious agents within the body is a new one and might become of importance in the problem of diet therapy if the relationship suggested by the authors proves to be true.

GALACTOSEMIA ASSOCIATED WITH CATARACTS IN AN INFANT

Galactosemia is an anomaly of carbohydrate metabolism in which the metabolism of galactose is impaired and this sugar accumulates in the blood in excessive amounts and may be excreted in the urine. This rare metabolic error has been described in infants and is obviously more likely to be detected during the period in which a child is primarily dependent for food upon milk with its accompanying lactose. The lactose, upon hydrolysis, gives rise to the galactose. The metabolic defect is of considerable interest in view of certain well recognized toxic effects of the monosaccharide galactose in experimental animals.

E. Bruck and S. Rapoport (*Am. J. Dis. Child.* **70**, *267* (*1945*)) have reported detailed studies on a case of galactosemia occurring in a 7 week old infant. The child was an undernourished male with a history of diarrhea and vomiting which had been partly controlled by feeding a dilute milk formula. He exhibited bilateral nuclear cataracts and an enlarged liver. He was lethargic and the tendon reflexes were absent, indicating pathologic changes in the nervous system. The urine contained a sugar which was demonstrated to be galactose, the fasting blood sugar was elevated to above 200 mg. per 100 ml. on several occasions, and about one half of this sugar was galactose. Galactose was present in the spinal fluid. Other routine chemical studies on the blood were not revealing except for a moderate lowering

of the serum proteins and an elevation of phosphatase. The child was anemic and there was albuminuria.

When the child was given a lactose- and galactose-free formula consisting of casein hydrolysate with added dextrose and polymers of dextrose, the following changes occurred: The urinary sugar and nonfermentable blood sugar disappeared within twenty-four hours, the albuminuria cleared within seventy-two hours, vomiting ceased, and a rapid weight gain ensued, the reflexes returned and the lenticular opacities (cataracts) disappeared. The liver slowly returned to normal size but remained more firm than usual. When milk was reintroduced into the diet a relapse was produced.

A series of investigations on the effects of the ingestion of galactose or glucose upon these sugars in the blood were made. The results may be summarized by stating that following the ingestion of galactose a fall in blood glucose occurred simultaneously with the rise of blood galactose. Furthermore, while the infant was on a milk diet the intravenous administration of glucose resulted in a lowering of the blood galactose level. The investigators interpret these results to signify that the two carbohydrates, galactose and glucose, are mutually antagonistic and that they may act in a competitive manner in some metabolic mechanism which resides in the liver. C. F. Cori (*Proc. Soc. Exp. Biol. Med.* **23,** *290* (*1926*)) demonstrated a similar competition between these sugars in absorption. There is additional indirect experimental evidence of competition of these sugars in the metabolism of the lens of the eye. Thus, it is well recognized that cataracts are often associated with abnormalities of carbohydrate metabolism such as diabetes mellitus and alloxan diabetes (C. C. Bailey, O. T. Bailey, and R. S. Leech, *New Engl. J. Med.* **230,** *533* (*1944*)). In 1935 H. S. Mitchell and W. M. Dodge (*J. Nutrition* **9,** *37* (*1935*)) described cataracts, growth retardation, and other changes in rats fed a diet containing 70 per cent of lactose. It was then demonstrated that the cataracts developed even more rapidly on a diet containing galactose (H. S. Mitchell, *Proc. Soc. Exp. Biol. Med.* **32,** *971* (*1935*)), that the feeding of either of these sugars resulted in a pronounced elevation of the blood sugar of the rats, and that galactose feeding, which caused the cataracts to develop in a shorter time, produced the higher blood sugar level (P. L. Day, *J. Nutrition* **12,** *395* (*1936*)). W. J. Darby and P. L. Day (*J. Biol. Chem.* **133,** *503* (*1940*)) found that at least one other sugar, the pentose xylose, when fed to young rats as 35 per cent of the diet, resulted in the development of cataracts and in certain other toxic manifestations. The blood sugar levels of the xylose fed rats were lower than those of control galactose fed rats although cataract development was slightly more rapid in the xylose fed group. These facts led the latter investigators to point out that both of the cataractogenic sugars, xylose and galactose, are to be considered as derivatives of d-threose while the noncataractogenic sugars tested are derivatives of d-erythrose, and to suggest that the toxic effect of the former sugars is related to their configurations. Whether this effect is due to an inhibition of carbohydrate metabolism in the lens by excessive amounts of these sugars remains hypothetical, although it is known that galactose is directly toxic for lens tissue (D. B. Kirby, K. Estey, and R. von E. Wiener, *Tr. Am. Acad. Ophth. Otolaryn.* **37,** *196* (*1932*)). The several evidences of competition between glucose, which is readily metabolized by normal tissues, and galactose, which is less readily metabolized by tissues, may indicate that under special conditions excessive quantities of galactose may be made to interfere seriously with normal oxidation of glucose. It is reasonable that xylose and galactose act similarly due to their configurational relationships.

It is generally believed that the liver is the

site of conversion of galactose to glucose. In the reported cases of galactosemia there are indirect evidences of impaired liver function. Thus it seems that this error of metabolism is basically a decreased rate of conversion of galactose to glucose by the liver which results in an accumulation of galactose in the blood and, finally, thereby the appearance of toxic manifestations due to the local presence of excessive galactose at the tissues. It appears possible that the cataract in the case described by Bruck and Rapoport (*loc. cit.*) may have been analogous to the experimentally produced galactose cataract. This view is supported by the remarkable improvement which resulted from merely removing galactose (as lactose) from the diet of the infant.

It is to be emphasized that galactosemia is a rare condition and that the toxic effects of lactose and galactose as discussed in this review have no known significance for the feeding of normal infants. The general interest in this inborn error lies in the insight which it may give into the metabolism of commonly ingested foodstuffs.

METABOLIC OXIDATION OF SULFIDE

For a quarter of a century there has existed evidence suggesting that sulfide sulfur can be oxidized to sulfate in the body. H. W. Haggard (*J. Biol. Chem.* **49**, *519* (*1921*)) showed that oxygenated blood or oxygenated plasma can oxidize sulfide sulfur, and that large amounts of sulfide can be given by repeated slow intravenous administration to the dog without apparent ill effect. From the latter finding he concluded that the rate of oxidation of sulfide in the intact animal may be high enough that many times the lethal amount can be handled without serious injury. W. Denis and L. Reed (*Ibid.* **72**, *385* (*1927*)) also obtained evidence that administration of sodium sulfide to the dog is followed by some oxidation to sulfate, and found that after feeding elementary sulfur to dogs about 10 per cent of it appeared in the urine as sulfate and neutral sulfur. J. C. Andrews (*Ibid.* **122**, *687* (*1938*)) demonstrated that sulfur can be converted into sulfide *in vitro* by the action of the intestinal flora of the dog and this would appear to be the first in a series of changes by which ingested sulfur is transformed to sulfate.

A study of the oxidation of sulfide sulfur has been reported by D. D. Dziewiatkowski (*J. Biol. Chem.* **161**, *723* (*1945*)), using the radioactive isotope S^{35} to label the sodium sulfide administered to rats. Male albino rats about 5 months old were used in the study. Two rats fed Purina dog chow were first given 2 ml. of a solution of sodium sulfide containing approximately 1.7 mg. of sulfide sulfur immediately after one of their daily feeding periods of thirty minutes. For the following twenty-four hours the urine and feces were collected with as little cross contamination as possible. The rats were then anesthetized with nembutal, their hair pulled out for analysis, the carcasses dissected, and separate tissues weighed and analyzed.

The urine was analyzed for inorganic sulfate, total sulfate, and total sulfur, each fraction being isolated as barium sulfate. Aliquots of tissues and feces were hydrolyzed for eight to twelve hours with 10 per cent sodium hydroxide on a steam bath, oxidized by melting with Na_2CO_3 and Na_2O_2, and the sulfur separated as barium sulfate after dissolving and acidifying the melt.

For the 2 rats 94.7 and 64.5 per cent of the radioactive sulfide dose was recovered respectively. Relatively small amounts (2 to 6 per cent) were recovered from the feces and gastrointestinal tract. The urinary total sulfate contained 49.1 and 48.2 per cent, and the nonsulfate sulfur 29.4 and 3.5 per cent respectively of the dose. Al-

though the latter differences are unaccounted for, the experiment does appear to establish that almost half of a dose of 1.66 mg. of sulfur as sodium sulfide given to a young adult rat is oxidized to sulfate in twenty-four hours.

In a second experiment the sodium sulfide was injected intraperitoneally, and the excretion of the sulfur was followed in the urine and the feces for six days. The results supported those of the earlier experiment, without any wide divergence between the 2 animals. During the first twenty-four hours after injection the 2 rats showed a urinary excretion of 61.1 per cent of the dose as total sulfate, and 64.9 and 63.6 per cent respectively as total sulfur. The feces contained 16.3 and 13.5 per cent respectively. On subsequent days the total urinary sulfur had dropped to very low levels. It appeared that following intraperitoneal injection of 0.2 mg. of sulfur as sulfide to young adult rats about 80 per cent of the sulfur is excreted in one day, and about 90 per cent in six days. It is not obvious why a greater fraction of the dose should appear in the feces after intraperitoneal injection than after oral administration.

Other points of interest noted by Dziewiatkowski are (1) that small fractions of the ingested doses were recovered from all the tissues analyzed, including the hair, and (2) that part of the radioactive sulfur of the urine was present as ethereal sulfate. The amount appearing in the ethereal sulfate fraction was increased by the oral administration of phenol.

The results reported appear to confirm that sulfide can be oxidized in the body, and also to establish that a large fraction of the doses of sulfide given can be oxidized to sulfate with remarkable rapidity. The general conclusions are in this case not seriously questioned by the small number of animals employed in the study.

FATTY ACID OXIDATION

Within the last three years L. F. Leloir and J. M. Muñoz (*J. Biol. Chem.* **147,** *355* (*1943*); **153,** *53* (*1944*)), have demonstrated that an enzyme preparation from guinea pig liver, with suitable conditions, has the ability to oxidize butyrate. These conditions include the presence of adenylic acid, cytochrome c inorganic phosphate, magnesium ions, and an oxidizable substrate such as a 4 carbon dicarboxylic acid or glutamic acid. A. L. Lehninger (*Ibid.* **157,** *363* (*1945*)) reported later that adenosine polyphosphates or compounds capable of phosphorylating adenylic acid are required to activate the oxidation of saturated fatty acids by crude rat liver homogenates. The acids found to be oxidized were the straight-chain compounds having from 4 to 18 carbon atoms. The detailed experiments described in this paper led the author to postulate the working hypothesis that phosphorylation of the fatty acids takes place prior to their oxidation. He has now described further experiments (*Ibid.* **161,** *437* (*1945*)) made with a more purified enzyme preparation, and a close correlation is apparent between his results and those of Leloir and Muñoz. Lehninger has also introduced the term co-oxidant to designate the second substrate (fumarate, alpha-ketoglutarate, etc.) which must be oxidized in the presence of adenylic acid in order to initiate or support the oxidation of the fatty acid.

The enzyme suspensions were prepared by removing the liver of a newly killed rat, chilling, cutting into pieces, and homogenizing in two parts of a cold buffer solution containing sodium phosphate, sodium chloride, potassium chloride, and magnesium sulfate. The homogenate was filtered through cheesecloth, centrifuged four times,

and each time resuspended in the cold buffer. Such a preparation has little or no endogenous activity and is therefore better adapted for the study of the activation than cruder homogenates or the preparations of Leloir and Muñoz. It is capable of oxidizing fatty acids having more than 4 carbon atoms in the presence of adenosine triphosphate, or of oxidizing octanoic acid in the presence of adenylic acid if the simultaneous oxidation of a suitable co-oxidant occurs.

The oxidation of octanoate was demonstrated by use of a Warburg respirometer, using a system comprised of the enzyme suspension, adenylic acid, magnesium sulfate, phosphate buffer, and cytochrome c. Increased oxidation was caused by addition of succinate, malate, oxalacetate, citrate, or pyruvate, as measured both by the increased oxygen utilization and the formation of acetoacetate. When succinate was used as the co-oxidant, the addition of malonate completely blocked succinate oxidation, and with it the oxidation of octanoate. When alpha-ketoglutarate was added, it was completely oxidized to succinate simultaneously with octanoate oxidation.

It is of interest that although alpha-ketoglutarate and adenylic acid would mediate the oxidation of 0.007 M octanoate, adenosine triphosphate would not mediate the oxidation until the concentration of octanoate was decreased to 0.001 M, which is of the order of concentration of free fatty acids in normal tissues. The specificity of adenosine triphosphate for the activation was great; it could not be replaced, in the absence of a co-oxidant, by adenosine diphosphate, adenylic acid, phosphopyruvic acid, thiamine pyrophosphate, or sodium pyrophosphate. Evidence is adduced which suggests that in the alpha-ketoglutarate-adenylic acid system, a part of the octanoate is oxidized by reactions involving a phosphorylated intermediate which arises during alpha-ketoglutarate oxidation but does not necessarily act through the adenylic acid.

The activation of the oxidation of octanoate by adenosine triphosphate was found to be inhibited to a considerable extent by small concentrations of iodoacetate, iodoacetamide, p-chloromercuro-benzoate, potassium ferricyanide, o-iodosobenzoic acid, and iodine. These reagents inhibit many enzymes requiring a free sulfhydryl group for activity, suggesting that one of the enzymes involved contains such a group. The rate of oxidation by the enzyme in the presence of adenosine triphosphate increases with increasing length of the carbon chain of the fatty acid, being over three times as great for octanoic as for butyric acid, whether judged by oxygen uptake or acetoacetate production. If the enzyme preparation was "aged" at 0°C. for three or four hours, it was no longer active with the usual supplements, but regained nearly all of the lost activity on the addition of diphosphopyridine nucleotide, indicating that this compound is also involved in the oxidation mechanism.

These experiments appear to prove that phosphorylation of fatty acid by adenosine triphosphate is necessary before oxidation, and that the phosphorylation may be coupled with the oxidation of alpha-ketoglutarate. The exact nature of the phosphorylated fatty acid has not been reported. Fatty acid oxidation thus appears to bear a close analogy to that of glucose. Extending this analogy further, it seems probable that fatty acid oxidation, yielding considerable energy, probably also results in the formation of energy-rich phosphate compounds for vital functions.

MALNUTRITION IN JAPANESE PRISON CAMPS

Despite the clinical reports on prisoners of war from Japanese camps and prisons, there is still lack of information on the pathogenesis of neurologic lesions which

occur in the various forms of malnutrition. G. C. Dansey-Browning and W. M. Rich (*Brit. Med. J.* **1**, *20* (*1946*)) have again called attention to the frequent occurrence of retrobulbar neuritis in prison beriberi. Their experience was with 30 released prisoners most of whom had suffered from beriberi or dysentery, and some malaria. During the late stages of beriberi, most of the men had noted failing vision. Ten of the subjects had central scotomata, there was bilateral pallor of the optic discs in every case, and 4 cases showed primary optic atrophy.

A review of the Japanese medical writings on beriberi (R. H. Elliott, "Tropical Ophthalmology," *p. 405, Frowde, London* (*1920*)) indicated that retrobulbar neuritis, scotomata, oculomotor palsies, and diminished corneal and conjunctival sensitivity have been encountered with varying frequency in Oriental beriberi. Because of the great similarity between the scotomata observed in liberated prisoners with beriberi and those found in instances of poisoning by tobacco, alcohol, lead, and quinine, Dansey-Browning and Rich believe there may be a common pathogenesis. The evidence for this is still indirect, and conclusive proof is made difficult since the period between onset of visual difficulty and irreversible nerve changes may be so short that therapy with the suspected missing nutrient is usually exhibited too late for amelioration of the optic nerve lesion.

M. M. Musselman (*War Medicine* **8**, *325* (*1945*)) has also witnessed severe nutritional deficiencies among American prisoners captured in Bataan. During the Bataan campaign itself the food issue was reduced to as low as 900 calories. The ensuing forced marches without food, and the prison diet of 1500 calories containing only 30 g. of protein, led to beriberi in about half the prisoners. The beriberi was characterized by neuritis, edema, bradycardia, and oliguria.

Signs of pellagra did not appear for four to eight months, but progressed rapidly. Oral and dermal lesions became ulcerated and infected in a few weeks. Diarrhea was widespread, but the role of pellagra could not be distinguished from that of the infectious dysenteries. After depletion of a small stock of compressed yeast tablets, a culture of wild yeast was grown, but there was never enough to treat any but the worst cases showing infections or severe neuritis.

Many of the neurologic and optic signs of beriberi described by Dansey-Browning and Rich were noted. Visual disturbances ranged from complete blindness to mere blurring of vision. Cardiac symptoms of beriberi consisted of palpitation, pain, and dyspnea, but the heart rate was sometimes rapid. There was often a curious inflammatory lesion on the skin of the leg. Ureteral colic was frequent and uric acid or mixed calculi were common. There were also a number of cases with stones in the parotid or submaxillary ducts.

The number and variety of signs and symptoms make it difficult to correlate these with specific deficiencies, and these are no doubt conditioned by many other factors, but the severe diseases caused by extreme malnutrition were all too obvious in Japanese prison camps.

BRITISH ANTI-LEWISITE (BAL)

Both in Great Britain and this country considerable research effort in biochemical laboratories during the war years was directed to a search for antidotes for war gases. The vesicant gas lewisite, developed during the close of World War I but not employed in combat, as well as mustard gas, appeared to be likely chemical warfare agents for use in the past war. Two principal reports on the development of an

antidote for lewisite have appeared, the first from England by R. A. Peters, L. A. Stocken, and R. H. S. Thompson (*Nature* **156,** *616 (1945)*), and the second a compilation of American studies by L. L. Waters and C. Stock (*Science* **102,** *601 (1945)*). The first report deals chiefly with the background for discovery of the antidote which is 2, 3 dimercaptopropanol (British Anti-Lewisite or BAL), and the latter principally with the use and testing of the new compound. The discovery of this compound has a special interest since it was based on the same technics and knowledge of *in vitro* metabolism which have proved so fruitful in elucidating the biochemical functions of the vitamins. In fact the English workers emphasize that the discovery of BAL is an important advance in the theory that a "biochemical lesion in a tissue formed by the partial blocking (as by the partial deficiency) of an enzyme system can cause pathological damage."

Lewisite is an organic trivalent arsenic compound with strong vesicant properties. As a background for the theory that trivalent arsenic compounds might affect tissue enzyme systems were the reports of C. Voegtlin, H. A. Dyer, and C. S. Leonard (*Pub. Health Reports* **38,** *1882 (1923)*), F. G. Hopkins and M. Dixon (*J. Biol. Chem.* **54,** *527 (1922)*), and S. M. Rosenthal *Pub. Health Reports* **47,** *241 (1932)*). These reports made it probable that the action of arsenicals is related to their ability to combine with sulfhydryl groups of the tissue, and the essential nature of intact sulfhydryl groups for activity of several enzymes has been proved repeatedly. R. A. Peters had reported in 1936 (*Nature* **138,** *327 (1936)*) that the vesicant dichlorodiethyl-sulfone had a selective action on the ability of brain tissue to oxidize pyruvate, and postulated that lewisite might act in a similar manner. This selective action of lewisite and arsenite in low concentrations was demonstrated, and the probability that the effect was related to the *in vivo* toxicant action was strengthened by the finding that blood pyruvate levels were increased in arsenite poisoning. The toxic action of arsenic compounds was therefore likened to thiamine deficiency; in the former case the protein component of the pyruvate oxidation system would be inhibited, in the latter there is a lack of the coenzyme component of the system. The dependence of the pyruvate enzyme system on intact sulfhydryl groups was demonstrated, and the possibility of reversing the combination of lewisite and tissue sulfhydryl was encouraged by the finding of A. Cohen, H. King, and W. I. Strangeways (*J. Chem. Soc. (London), Part* **II,** *3043 (1931)*) that such thioarsenites were dissociable.

The search for a lewisite antidote was therefore guided by testing of thiol compounds *in vitro* on the inhibited pyruvate oxidase system, a test system which according to the investigators proved entirely reliable. However, many thiol compounds were tested without finding a promising antidote, and not at all encouraging were the findings that the thioarsenites resulting from combination of arsenicals and thiol compounds could be as toxic as the original arsenicals. Other experiments on the combination of lewisite with the reduced form of the protein keratin, showed that the lewisite formed the most stable compounds when combined with two thiol groups of the protein. Hence the search was directed particularly toward dithiol compounds which might compete successfully with thiol groups in the tissue for the toxic lewisite. Of the simple dithiol compounds tested, 2, 3 dimercaptopropanol (BAL) proved to be active and suitable for skin treatment. BAL gave nearly complete protection against the inhibition of pyruvate oxidation caused by lewisite, and it neutralized the lewisite inhibition of skin respiration. Tissue cultures which were poisoned with lewisite recovered upon addition of BAL, demonstrating a direct antidotal action on the cells.

Toward practical application, the British workers demonstrated (1) that animals treated as long as two hours previously with lewisite, and seriously poisoned, could be saved by application or injection of BAL, and (2) that vesication by lewisite in human subjects could be prevented by application of BAL as long as an hour after lewisite contamination. An increased rate of excretion of arsenic after administration of BAL pictures an active withdrawal of arsenicals from vital tissue components and elimination of the product.

Work in America, compiled by L. L. Waters and C. Stock (*loc. cit.*), involved an intense program, coordinated through several governmental agencies, on the preparation and manufacture, biochemistry, toxicology, therapeutics, and clinical application of BAL. Biochemical work amply confirmed that the trivalent arsenicals exert their action by combination with sulfhydryl groups, and that BAL can prevent or reverse this action. The greater affinity of arsenic for BAL than for tissue sulfhydryl was demonstrated. Thus when trypanosomes or spermatozoa which had been subjected to lethal concentrations of arsenic were treated with BAL, they regained their motility and normal cytologic appearance. In a Canadian report (L. Young, *Science* **103,** *439* (*1946*)) BAL containing radioactive sulfur has been described. The rate of skin penetration, distribution, and urinary excretion has thus been studied. BAL does not appear to accumulate preferentially in any of the major organs. Approximately 80 per cent of the radioactive sulfur administered intramuscularly is excreted in twenty-four hours, but apparently only a small proportion of this is in the form of unchanged BAL.

American studies on the clinical use of the new drug has led to BAL eye solution, eye ointment, an ointment for skin application, and a benzyl benzoate-peanut oil solution for intramuscular injection. As a result of enzyme studies demonstrating that the toxic action of other metals may also be due to their effect on tissue sulfhydryl, BAL may prove useful for example in mercury poisoning. Favorable clinical results have been obtained in mercury poisoning as well as in the toxic manifestations of arsenotherapy of syphilis.

BAL is itself rather toxic and this may be related in part to its strong reducing properties. It can destroy hemin or oxyhemoglobin by opening the porphyrin ring, and reduce methemoglobin or cytochrome c. Modified formulas may be expected, and already a report by J. F. Danielli and coworkers (*Nature* **157,** *217* (*1946*)) describes a glucoside of dithioglycerol (BAL-Intrav) which may be more suitable for systemic arsenic poisoning.

VITAMIN B-COMPLEX IN THE COLOSTRUM AND MILK OF RUMINANTS

A decade ago extensive studies of the very high carotene and vitamin A concentrations in the colostrum of the cow (W. J. Dann, *Biochem. J.* **27,** *1998* (*1933*)) and in human colostrum (*Ibid.* **30,** *1644* (*1936*)) were made. Similar studies with other species have been made. Until the investigation of P. B. Pearson and A. L. Darnell (*J. Nutrition* **31,** *51* (*1946*)), only scattered observations on the concentrations of the individual members of the vitamin B-complex in colostrum and milk have been reported. These workers determined the mean values and the standard deviations for thiamine, riboflavin, nicotinic acid, and pantothenic acid in the colostrum and the milk of the ewe and the cow. A definite species difference in the concentrations of thiamine, riboflavin, and nicotinic acid in both the colostrum and milk was observed. The colostrum and the milk of the ewe contained substantially higher amounts of

these three vitamins than the corresponding secretions of the cow. In both species the concentrations of thiamine and riboflavin in the colostrum were significantly higher than those in the milk. In contrast, the pantothenic acid content of the colostrum of both the cow and the ewe was significantly lower than in the milk. There was no significant difference between the nicotinic acid levels of the colostrum and the milk of the cow, while the nicotinic acid content of ewe's milk was significantly higher than in the colostrum. It was interesting to note that the high level of thiamine in the colostrum of the cow was maintained for at least nine days but had dropped to the normal level in milk by the thirtieth day. The high level of riboflavin in cow's colostrum declined rapidly after the first day and approached the normal level for milk around the seventh day after parturition. There was a marked increase in the pantothenic acid concentration on the second day, and by the third day it had reached the normal value for milk.

These authors suggested that an interesting association between the rate of growth which is characteristic of a species and the concentrations of the B-complex vitamins in the colostrum and the milk might exist. It was pointed out that the newborn lamb doubles its weight in approximately fourteen days, while the newborn calf requires about forty-seven days. The higher levels of thiamine, riboflavin, and nicotinic acid in the colostrum and milk of the ewe may be necessitated by greater growth demands in the lamb. It was stated that limited observations on the concentrations of these vitamins in human and horse milk indicated that the average values were much lower than the corresponding ones in cow's milk. These studies are of special importance in the field of comparative nutrition and may point to possible ways by which nutrition aids in maintaining the growth potential which is characteristic of the species.

THYROXINE SYNTHESIS BY THYROID TISSUE

During the last four years I. L. Chaikoff and his collaborators in an interesting series of papers have described the application of the tracer technic to the study of the synthesis of thyroxine by thyroid tissue. M. E. Morton and I. L. Chaikoff (*J. Biol. Chem.* **147,** *1* (*1943*)) demonstrated that when radio-iodide containing I^{131} was added to bicarbonate-Ringer's solution in which surviving thyroid slices were incubated for some hours, the presence of radio-diiodotyrosine and radio-thyroxine could be demonstrated. The conversion of iodide to these two compounds was shown to occur in slices from the thyroid glands of the sheep, dog, and rat. The greatest conversion was observed with rat tissue in which after three hours of incubation, 60 to 70 per cent of the radio-iodide was converted to diiodotyrosine by 350 mg. of rat thyroid tissue, and as much as 12 per cent were converted into thyroxine. With sheep and dog thyroid slices the rate of conversion was about half as great. The ability of sheep and rat tissue to perform this conversion was lost if the tissue was disrupted by homogenization.

A. L. Franklin and I. L. Chaikoff (*J. Biol. Chem.* **152,** *295* (*1944*)) extended this work by following up the reports of several investigators that administration of some sulfonamides caused a hypertrophy of the thyroid gland. The compounds diminished the production of thyroid hormone, although the evidence produced did not indicate whether the sulfonamides might have acted by interfering with the selective removal of iodide from the blood stream by the thyroid, or by affecting the release of thyroxine into the blood stream.

By the application of the technic developed by Morton and Chaikoff, the effects of some sulfonamides on the conversion *in vitro* of iodide to di-iodotyrosine and thyroxine by surviving slices of thyroid tissue of the cow, sheep, and dog were examined. It was found that the ability of the slices to remove I^{131} from the Ringer's solution was not significantly decreased by the presence of 10^{-3} M sulfanilamide. On the other hand the incorporation of radioiodide into di-iodotyrosine and thyroxine was greatly inhibited by sulfanilamide, sulfapyridine, sulfaguanidine, or sulfathiazole. It appears therefore that the sulfonamides exert their action on the thyroid gland by inhibiting one or more reactions occurring during the transformation of inorganic iodide into di-iodotyrosine.

A. L. Franklin, I. L. Chaikoff, and S. R. Lerner (*J. Biol. Chem.* **153,** *151 (1944)*) next applied the same technic to a study of the activity of a number of other goitrogenic substances. They observed that thiourea, thiouracil, thiocyanate, para-aminobenzoic acid, and para-aminophenylacetic acid (10^{-3} M) strongly depressed the conversion of inorganic iodide into di-iodotyrosine and thyroxine by surviving sheep thyroid slices. Allythiourea inhibited the change effectively at 10^{-4} M, but methyl cyanide had no effect at concentrations up to 10^{-1} M. Thiourea, thiouracil, and para-aminobenzoic acid at 10^{-3} M did not have any significant effect on the ability of the thyroid slices to take up the iodide present in the medium, but thiocyanate was inhibitory in this regard.

A. Taurog, I. L. Chaikoff, and A. L. Franklin (*J. Biol. Chem.* **161,** *537 (1945)*) have attempted to define the molecular specificity of compounds similar in structure to the sulfonamides which are able to inhibit the *in vitro* conversion of inorganic iodide to thyroxine by thyroid slices. Forty compounds were tested, and certain points were established. A free aromatic amino or free aromatic hydroxyl group tends to increase inhibitory activity, and blockage of the amino group by acetylation generally reduces inhibitory activity. The sulfonamide and carboxyl groups do not confer any inhibitory activity. Among the isomers of aminobenzene sulfonamide and aminobenzene sulfonic acid, the ortho compounds were least active as inhibitors. The results also indicated that there is a correlation between the ease with which the compounds tested could be oxidized, and their inhibitory activity.

It is interesting to compare the results of Taurog *et al.* with those obtained by a different technic. E. B. Astwood (*J. Pharmacol. Exp. Therap.* **78,** *79 (1943)*) tested upwards of one hundred compounds for goitrogenic activity by administering them orally for ten days and measuring the degree of enlargement of the thyroid and proliferation of its acinar cells. Those compounds observed by Astwood to be goitrogenic were also found by Taurog *et al.* to inhibit the conversion of iodide to di-iodotyrosine and thyroxine. In addition to this, many compounds not observed to produce gross or histologic changes in the thyroid when fed to the rat did however inhibit the formation of di-iodotyrosine and thyroxine by thyroid slices *in vitro*. The suggestion is made by Taurog *et al.* that this discrepancy would be resolved on the assumption that such compounds are modified or changed in the intact animal before reaching the thyroid, thus having no opportunity to affect it.

VITAMIN K AND TOOTH DECAY

At present little is known of the relation of vitamin K (menadione) to the development and maintenance of the teeth. H. Granados and H. Dam (*J. Dental Research*

24, *137* (*1945*)) reported preliminary observations on the relation of vitamin K deficiency, as produced by a diet containing 1 per cent sulfadiazine, to the growth, enamel pigmentation, and the rates of eruption and attrition of the incisor teeth of the white rat. A definite vitamin K deficiency was produced in this manner since the prothrombin time of the rats which received no vitamin K was between one hundred and ten and three hundred and sixty seconds, in comparison with thirty-three seconds in those rats which received a supplement of 1 mg. per cent of menadione. The incisors of all rats which received diets containing sulfadiazine showed a striking reduction in the rates of growth, eruption, and attrition and a decrease in enamel pigmentation. The vitamin K supplement had no effect on these abnormalities.

The effects of prolonged ingestion of synthetic vitamin K in rats have been studied by E. H. Hatton, A. Dodds, H. C. Hodge, and L. S. Fosdick (*J. Dental Research* **24,** *283* (*1945*)). In one experiment, low levels of synthetic vitamin K (2-methyl-1,4-naphthoquinone or the sodium bisulfite addition product) from 0.005 to 0.125 per cent of the diet, were fed for three generations. The diet used was a balanced, commercial mouse ration. On the basis of the food consumption records, it was calculated that the rats in the low and high vitamin K groups each ingested 1 mg. and 25 mg. respectively per day. No appreciable differences in rate of growth nor pathologic changes were observed in any of the three generations. No differences in the incidence of tooth decay were observed between the control and vitamin K supplemented animals.

In the second experiment, the Hoppert-Webber-Caniff caries-producing ration containing 60 per cent of coarse corn meal was supplemented with 0.1, 0.3, and 0.8 per cent synthetic vitamin K. The diet which contained 0.1 per cent vitamin K produced little, if any, change in growth rate, while the diets containing 0.3 and 0.8 per cent produced measurable reductions in the growth rate. However, there was again no reduction in the dental caries incidence when these large amounts of vitamin K were administered.

These studies on rats were preliminary to studies of the effect of vitamin K upon the incidence and progress of dental caries in man (D. Y. Burrill, J. C. Calandra, E. B. Tilden, and L. S. Fosdick, *J. Dental Research* **24,** *273* (*1945*)). For this experiment, 119 subjects were selected, each of whom had a greater than average susceptibility to tooth decay, yet had at least twelve unfilled proximal surfaces in positions where decay was likely to occur. These subjects were divided into two groups which were as nearly alike as possible in caries susceptibility. All subjects were instructed to chew one pellet of gum for ten or more minutes after each meal. No attempt was made to control the composition nor the quantity of food consumed by the experimental subjects. The gum given to the first group of 55 subjects was a regular commercial product. That given to the second group was a similar kind of gum, except that 0.75 mg. of the sodium bisulfite addition product of 2-methyl-1,4-naphthoquinone had been incorporated into each pellet. Since it was found that the quinone was soluble in the gum base and not readily released in the saliva, the compound was placed in the sugar coating of the gum pellets. The flavor of the gum was not altered by this addition and neither the subjects nor the examiners knew which gum had been given to any subject. Due to lack of cooperation 10 of the subjects in the plain gum group and 9 in the vitamin K gum group had to be dropped. A control group of subjects was selected from the same student classes as the two experimental groups. The members of this group were examined as carefully as those in the other two groups but were given no gum. They served only as a reference group for the comparison of the dental caries increments experienced by the experimental groups.

A reduction in the incidence of new carious

lesions was observed in both experimental groups. At the end of the eighteen month experimental period on the basis of only radiographic examination, it was stated that the incidence of new carious lesions in the subjects who had chewed vitamin K gum was 69 per cent lower than that of the control group. The incidence of new carious lesions in those subjects who had chewed plain gum was stated to be 60 per cent lower than that of the control subjects. On the basis of clinical and radiographic examinations combined, the increment of new caries in vitamin K gum subjects was calculated to be 54 per cent lower while the increment of new caries in the plain gum group was found to be 21 per cent lower than that of the control subjects. There was stated to be no apparent relation between the chewing of either gum and the inhibition of the progress of carious lesions which were present at the beginning of the experiment.

L. S. Fosdick, O. E. Fancher, and J. C. Calandra (*Science* **96**, *45* (*1942*)) have reported a definite reduction in the amount of acid produced in incubated saliva when synthetic vitamin K was present. W. D. Armstrong and J. W. Knutson (*Proc. Soc. Exp. Biol. Med.* **52**, *307* (*1943*)) and W. D. Armstrong, W. W. Spink, and J. Kahnke (*Ibid.* **53**, *230* (*1943*)) presented evidence which indicated that the antibacterial action of synthetic vitamin K was not specific but was a characteristic of a number of quinones which did not have vitamin K activity for animals.

Any experiment in which a reduction in the incidence of dental caries in man is achieved is of considerable interest to workers in the fields of dental and preventive medicine, but it is a serious omission when results are presented without any semblance of statistical evaluation. The presentation of the average caries increment for the groups studied and the percentage reductions in the caries increment of the experimental groups are without doubt the simplest means of recording the accumulated results. But such simple and inadequate mathematical evaluations, especially when applied to a disease with as many varied types of manifestations and etiologic causes as dental caries, hide the significance of the data obtained.

By multiplying the average number of new cavities per person presented by Burrill *et al.* by the number of persons in their respective experimental group, the total number of cavities observed by these workers in any group can be deduced. Thus one determines that the total number of new carious lesions observed at the end of eighteen months by radiograph in the 55 members of the vitamin K gum group was nine (0.17 x 55), while during the same period ten (0.22 x 45) new lesions were found in the 45 members of the plain gum group. The number of new cavities observed by clinical and radiographic examination was thirty-five (0.64 x 55) in the former group in comparison with fifty (1.10 x 45) new cavities in the latter group. In view of the difficulties encountered in making an accurate diagnosis of the initiation and extent of carious lesions, it is felt that these are extremely small differences. For example, D. F. Radusch (*J. Am. Dent. Assn.* **28**, *1959* (*1941*)) observed that when a subject was examined by three dentists in a study to determine the variability in oral diagnosis, the average variation was four and two-tenths carious teeth and five and eight-tenths carious surfaces.

The effect of synthetic vitamin K in chewing gum upon the incidence of dental caries has been investigated by the Medical Department Professional Service Schools, Washington, D. C. whose preliminary results are published as a note (*Bull. U. S. Army Med. Dept.* **5**, *265* (*1946*)). Sixteen men each chewed gum containing 1 mg. of synthetic vitamin K for at least ten minutes after consuming any food or liquid. A control group of 14 men chewed gum without vitamin K, for at least ten minutes after consuming any food or liquid. The men in

each group were examined clinically and by radiographs at intervals of three to four months. Analysis of the data obtained over the one year period indicated that there was no difference between the number of new carious lesions in the experimental and the control group.

If the data obtained in the experiment described by Burrill *et al.* are statistically significant and if the results can be repeated, the role of vitamin K in the maintenance of the erupted tooth may be of great importance. The question arises as to whether vitamin K is of nutritional or environmental importance in this problem. The human requirement for vitamin K is unknown at this time. The antibacterial activity of vitamin K has already been described by Fosdick *et al.*, but Armstrong and co-workers have presented evidence that indicates a general, nonspecific, antibacterial activity of quinones, independent of their vitamin K activity. At present it seems that the caries reduction described by Burrill and co-workers may have been due to an antibacterial effect of the synthetic vitamin K as a quinone and not as an essential nutrient.

A further point of interest about the effect of vitamin K in the maintenance of erupted teeth is the apparent difference between the ability to inhibit the initiation of carious lesions in man and in the rodent. Even with the large amounts of vitamin K fed to rats by Hatton and co-workers, there was no reduction in the caries incidence. Does this mean that the etiology of caries in the rodent is basically different from that of caries in man? The maximum incidence of caries in the white rat can only be obtained, so far as is known at present, by the use of a diet containing hard carbohydrate particles of the correct coarseness. This is one factor in which the etiology of caries in the rat differs from that of caries in man. The hamster and the cotton rat appear to differ sufficiently from the rat that particle size in these two species does not appear to be a determining factor in the initiation of carious lesions. This difference between the rat and these other species of rodents is sufficient to merit trials of the effect of vitamin K and other quinones on caries incidence in these other species. There is a great need in dental research for comparative experiments in the various species of animals which are susceptible to dental caries in an effort to classify the different types of carious lesion found experimentally and to determine which, if any, of these lesions have etiologic relations to carious lesions in man.

ALUMINA GEL AND THE ABSORPTION OF VARIOUS NUTRIENTS

There has been much speculation and a good deal of assumption concerning the effect of various therapeutic agents on the absorption of nutrients from the alimentary tract but comparatively little actual observation has been made. The report by W. S. Hoffman and H. A. Dyniewicz (*Gastroenterology* **5**, *512* (*1945*)) is therefore of interest.

These investigators state that, in view of the well known adsorptive and neutralizing properties of alumina gel ("Amphojel"), a diminished or delayed absorption of nutrient substance might well be postulated when this substance is given over a protracted period in the treatment of peptic ulcer. They set out, therefore, to determine whether in therapeutic doses alumina gel has any appreciable effect on the absorption of vitamin A as indicated by the levels of vitamin A in the blood following ingestion of this vitamin.

The vitamin A "tolerance curves" were determined (a) after ingestion of 75,000 I.U. of vitamin A, and (b) after intake of 1 ounce of alumina gel prior to ingestion of the same quantity of vitamin A. Similar observations in relation to the intake of aluminum phosphate gel ("Phosphaljel") were

made. Vitamin A tolerance curves in patients with peptic ulcer under control conditions and again after alumina gel had been taken every two hours during the waking hours for one or more weeks were also determined. Finally, a study of the fasting levels of plasma vitamin A in patients with peptic ulcer who had been on alumina gel management for at least two weeks was made.

The vitamin A was given as a concentrate of the natural esters of vitamin A which contained 200,000 I.U. per gram of cottonseed oil solution. The concentrate, diluted with corn oil and measured accurately into a small glass of orange juice, was ingested. Blood specimens at zero, three, six, and twenty-four hours after the ingestion of vitamin A were obtained. The tests with alumina gel were usually performed four days later. In some instances, however, the alumina gel tests were performed first and the control examinations four days afterwards. In the control patients no effort to regulate the diets was made. The patients with peptic ulcer were on a modified Sippy diet, alumina gel being used instead of Sippy powders. The blood samples were taken in the morning, the last dose of alumina gel having been given the evening before.

In the normal subjects the plasma vitamin A was found to range from 27 to 96 micrograms per 100 ml. with a mean of 53 and a standard deviation of 19. In these individuals four days later, after the administration of alumina gel, the plasma vitamin A levels ranged between 25 and 69 with a mean of 44 micrograms per 100 ml. and a standard deviation of 12. In the 17 subjects who were studied it was observed that the maximal plasma vitamin A concentration following the ingestion of this vitamin was diminished significantly when the ingestion was associated with the simultaneous administration of alumina gel. The peak of the blood vitamin A curve was delayed. Following the administration of aluminum phosphate gel only 6 cases were studied. Although this represented a group too small for statistical analysis, the results obtained suggested that the deviations attributable to this substance were slight.

In 7 patients who had been taking alumina gel in frequent doses for some time, little difference between the maximal rise of the blood level for vitamin A before or one or two weeks after taking the alumina gel was noted. The range of fasting plasma vitamin A concentration in 33 patients who were under treatment for peptic ulcer and who had been receiving alumina gel for at least two weeks and in many instances for a much longer time, was 19 to 91 with an average of 46. This mean was exactly the same as that found in normal persons.

These data indicate, therefore, that the vitamin A plasma level may be slightly depressed if alumina gel is given simultaneously with the vitamin or immediately before it. This depression, however, did not occur when alumina gel was given every two hours for one or two weeks before the tolerance curve was determined. Therefore it does not seem likely that the depressed absorption (if that is the cause of the flattened tolerance curve) is due to a tanning action on the intestinal mucosa. It seems much more likely that the action of alumina gel is due to its direct adsorbent effect. It appears, then, that in so far as vitamin A is concerned, the administration of alumina gel in the usual dosages is associated with effects which have little if any nutritional significance.

In a later investigation W. S. Hoffman and H. A. Dyniewicz (*Gastroenterology* **6**, *50* (*1946*)) describe the results of a study of the effect of alumina gel on the absorption of amino acids, ascorbic acid, glucose, and neutral fat from the intestinal tract. Tolerance curves were determined in hospital patients after oral or intragastric administration of a standard dose of the test substance. After an interval of about four days the test was repeated after 1 ounce of alumina gel had been given immediately

before the test substance. In some of the cases this procedure was reversed, the alumina gel experiment being performed first, the control some four days later. In all of the substances measured the tolerance curves were remarkably similar whether the alumina gel was given before the test substance was supplied. Although 27 out of 39 individuals showed lower maximal rises with alumina gel than in the control experiments the differences were not statistically significant.

The practical importance of these observations is in indicating that from the nutritional point of view the ingestion of alumina gel has no significant effect upon the availability of the commonly ingested foods. Even had the slight differences which were noted been statistically significant, they were so small that they would not appreciably affect the total quantity of absorbed nutrient substances. Thus, there appears to be no basis for the claim which has been made a priori without experimental test that the use of various absorbents as therapeutic agents may lead to nutritional deficiency.

INTERMITTENT FASTING AND LONGEVITY IN RATS

The effect of intermittent fasting upon the life span of rats has been studied by A. J. Carlson and F. Hoelzel (*J. Nutrition* **31,** *363 (1946)*). Intermittent fasting was begun at 42 days of age and was continued until death. The rats were fasted one day in four, one day in three, or one day in two. The control rats and the intermittently fasted rats when fed were supplied with food ad libitum. Three omnivorous and one vegetarian diet were used. The basic omnivorous diet consisted mainly of cooked, dried "whole veal" and starch, supplemented with yeast, cod liver oil, veal bone meal, and inorganic salt mixture. The second and third omnivorous diets consisted of the basic diet plus 10 per cent of crude fiber, as finely ground alfalfa stem meal or psyllium seed husks and specially cleaned kapok. The vegetarian diet consisted mainly of whole wheat flour, peanut flour, wheat gluten flour, corn gluten meal, linseed meal, supplemented with yeast, alfalfa leaf meal, and sodium chloride. The basic omnivorous diet contained about 35 per cent proteins, while the vegetarian diet contained about 30 per cent proteins. Lettuce trimmings were supplied daily as a supplement to all diets. The 137 white rats used in this experiment were distributed so that litter mates of the same sex were represented in the various degrees of fasting and in the various dietary groups.

With the exception of the females which were fasted only one day in four, the average life span of all groups of intermittently fasted rats exceeded that of the controls. The life spans of the males were, on the average, increased more than the life spans of the females. In 30 litter mate comparisons, the average life span of the males was increased ninety days by intermittent fasting; in 45 litter mate comparisons, the life span of the females was increased only twenty-three days. Fasting one day in four and one day in two seemed to be complicated by more extraneous factors than fasting one day in three. The earliest deaths of both males and females occurred in the groups which were fasted one day in four. Fasting one day in two appeared to be too strenuous a regimen for some of the rats when begun at the age of 42 days. One of the females in this group apparently died of hemorrhage from a chronic duodenal ulcer which is of relatively rare occurrence in rats. Some erosion and ulceration of the stomach were observed in other rats in these experiments but the lesions seemed to be due to lack of appetite and starvation associated with

respiratory infections. However, the lack of appetite accompanying respiratory infections was not associated with stomach lesions in many of the rats. The optimum amount of fasting for the average rat in this survey seemed to be one day in three. This degree of fasting increased the life span of the males about 20 per cent and that of the females about 15 per cent. Since the average lengths of the femurs from the fasted rats at death were equal to those of the controls, the intermittent fasting would appear to have increased the life span without stunting the growth rate of the rats.

Some interesting relations between hereditary constitution, pre-experimental condition of the rats, and the life expectancy were observed. Despite the use of four different diets and four different combinations of feeding and fasting, 67 per cent of the rats died between the ages of 550 and 850 days. These workers stated that some litter mates, after having been kept from four hundred to a thousand days on widely divergent nutritional regimens would die within a few days of one another. Rats from small litters lived considerably longer than those from large litters. The rats which were the heaviest at the beginning of the experimental period tended to live longer on any dietary regimen than the lighter rats.

Intermittent fasting decreased the rate of incidence and the rate of growth of mammary tumors, in proportion to the degree of fasting. These observations parallel those of A. Tannenbaum (*Am. J. Cancer* **38**, *335* (*1940*); *Cancer Research* **2**, *460* (*1942*)) who observed a positive relation between a reduction in the caloric intake and the retarded development of tumors in mice.

These studies on the relation of intermittent fasting to longevity differ in several ways from the experiments of C. M. McCay and associates in which with rats, the rate of growth was retarded by a continuous restriction in the caloric intake (*Nutrition Reviews* **2**, *3* (*1944*)). By this means it was possible to prevent the rats from reaching growth maturity for periods up to a thousand days. Then when the rations were fed ad libitum a period of rapid growth followed. Such periods of growth retardation resulted in smaller rats at growth maturity even when ample calories were supplied after the restricted period. In the intermittent fasting experiments of Carlson and Hoelzel there was no appreciable stunting of the growth rate as manifested by the length of the femurs. The caloric restriction method seemed to result in a greater increase in the life span than intermittent fasting, although the different strains of rats used by the two groups of workers and the different diets may have affected the results. McCay and associates concluded that the lower incidence of respiratory infections observed in the retarded rats was the immediate factor responsible for the increase in longevity. Carlson and Hoelzel did not state whether the incidence of respiratory infection was altered by the periods of intermittent fasting.

ENVIRONMENT AND HEREDITY IN RELATION TO VITAMIN CONTENTS OF PLANTS

The literature on the subject of the relation of environment and heredity to the vitamin content of plants is steadily growing, with contributions of fundamental interest as well as those with very practical applications.

In an extensive study of the influence of mineral nutrition, soil fertility, and climate on the carotene and ascorbic acid contents of turnip greens, L. Bernstein, K. C. Hamner, and R. Q. Parks (*Plant Physiol.* **20**, *540* (*1945*)) conducted two complete experiments, one in late spring and one in early fall at Ithaca, New York. The supplies of

macronutrients, calcium, magnesium, potassium, nitrate, phosphate, and sulfate were varied widely in sand cultures and to a lesser extent in soil plantings.

In turnip leaves the ascorbic acid content increases from the basal to distal ends of leaves and from oldest to youngest leaves. Carotene was distributed fairly uniformly throughout the leaf, and oldest leaves were only slightly lower in carotene content than younger leaves. Carotene appeared to decrease during the middle of the day. The middle leaves of plants were used for vitamin analysis.

In the spring experiment, average carotene values were higher and ascorbic acid contents much lower in field plots than in pot-cultures, whereas in the fall experiment, carotene values were approximately equal and ascorbic acid contents only slightly higher in field plots. The marked effects of environmental factors other than soil variables were apparent throughout these studies. Climatic influences, involving light and temperature relations, are important for these two vitamins. Variations in the supply of these macronutrient elements in sand cultures and variations in fertilizer practices in soils have little effect on carotene and ascorbic acid contents of turnip greens.

All plants which were low in carotene were also chlorotic owing to a nutrient deficiency. Phosphorus deficient plants produced little growth but the leaves were very green and high in carotene content. Thus carotene content is not necessarily directly correlated with growth. In soil-pot cultures differences in carotene could be attributed to differences between replications but not to nutrient treatments. Chlorophyll content was relatively high in plants given treatments deficient in phosphate or rich in nitrogen.

With respect to ascorbic acid content, differences in environment between locations and between dates of harvest have a distinct effect. Plants grown in soil showed no differences in ascorbic acid contents due to fertilizer treatments. In sand-cultures potassium deficient plants were low in ascorbic acid.

In a field study of the relation of environmental and hereditary factors to ascorbic acid content of cabbage grown in Wisconsin, F. G. Smith and J. C. Walker (*Am. J. Bot.* **33,** *120 (1946)*) found no definite correlation between the content of this vitamin and stage of head maturity, soil fertility, yield, amount of light received preceding harvest, temperature, or day length. Ascorbic acid content is usually lower in the white inner leaves than in the outer green leaves. Among varieties significant differences were found which occurred independently of environmental fluctuations. Varieties in early pointed groups were in general higher than those in round-headed groups. Mid-season flat and some late flat varieties were lowest in ascorbic acid content.

In a series of closely related strains there was a highly significant increase in one season over the previous one. The relative importance of genetic and environmental factors in their influence on ascorbic acid content of cabbage is a complicated question but the results reported here emphasize the significance of heredity. Two lines derived from a single plant and inbred for seven generations contained 47 and 52 mg. per 100 g. of tissue. A line from another plant, inbred five generations, produced 85 mg. per 100 g. Characters responsible for these contents were apparently well fixed and F_1 crosses between high and low lines resulted in intermediate levels. This is additional evidence that by hybridization, higher vitamin contents may be introduced into types already desirable because of other characters.

An interesting temperature-sensitive mutant of Neurospora has been discussed by H. K. Mitchell and M. B. Houlahan (*Am. J. Bot.* **33,** *31 (1946)*). Like other strains of this mold, it differs from the wild type parent by a single gene, as shown by recovery of the mutant in crossing experiments with known types. This mutant requires riboflavin and

this requirement is a function of temperature. At temperatures below 25°C. it grows and synthesizes riboflavin nearly as rapidly as the wild strain but above 28°C. it is necessary to supply the vitamin from an external source to obtain growth comparable to that normally obtained in a test period of eighty-four hours. However, if more time is allowed and a small "primer" of riboflavin provided, growth will continue spasmodically to produce a dry weight equal to the wild type. A limited capacity for synthesis of riboflavin must exist in this mutant over the temperature range from 25° to 43°C. This behavior may be explained on the assumption that the gene involved has not been completely inactivated but altered in such a way that it controls the production of a modified and temperature-sensitive enzyme responsible for the biosynthesis of riboflavin. Forthcoming results from this laboratory may be expected to elucidate this problem further. In an attempt to utilize this mutant for bioassay purposes, it was found that tissue extracts were inhibitory rather than stimulatory. This inhibition was reversible and shown also by lumichrome and lumiflavin.

G. W. Beadle and E. L. Tatum (*Am. J. Bot.* **32,** *678* (*1945*)) have outlined their methods of producing and detecting mutations in the mold, Neurospora, concerned with nutritional requirements. Among 68,198 single-spore strains, derived from material treated with x-rays, ultraviolet, or neutron bombardment, 380 strains with altered nutritional requirements were obtained. Many of these differed by single genes from the wild-type parent. Nutritional alterations concerned requirements for the B-vitamins, amino acids, and purine and pyrimidine bases. These genetic changes reflect the function of genes in determining enzyme specificities and thus exercise control over particular chemical reactions. No mutant types were found in which fat soluble vitamins are required for growth. Possibly these vitamins could not be utilized when supplied in the medium. The role of fat soluble vitamins or provitamins in plant physiology remains in need of much further study.

NUTRITIVE VALUE OF SUNFLOWER SEED MEAL

Sunflower seeds as a possible source of food of good nutritive quality have been almost entirely overlooked until recently. The Chinese have long eaten them as roasted nuts but only occasionally have they been utilized by western peoples. Recent increase in the production of the seed, mainly as a source of oil, makes a critical evaluation of the nutritive quality of the meal remaining after oil extraction, of particular interest.

Two important classes of nutrients, namely members of the B-vitamins and proteins of good nutritive value, are present in abundance in sunflower seed meal. H. G. Day and E. Levin (*Science* **101,** *438* (*1945*)) have shown that rats on a vitamin B-complex free basal diet to which 5 per cent sunflower seed meal was added, exhibited gains superior to those on the basal diet supplemented with 5 per cent wheat germ, corn germ, or soybean meal. The growth rates for a fourteen week period were 180, 70, 70, and 119 g. respectively. Rats receiving 5 per cent dried brewers' yeast showed somewhat larger gains than those on sunflower seed meal. Undetailed experiments indicated that pantothenic acid and riboflavin were the limiting growth factors for rats receiving sunflower seed meal.

Reports on the nutritive value of the proteins have been somewhat conflicting, possibly due to differences in the samples tested, and brought about by the severe heat treatment during extraction of the oil. H. H. Mitchell, T. S. Hamilton, and J. R. Beadles (*J. Nutrition* **29,** *13* (*1945*)) have shown that the meal processed at low temperature by solvent extraction contains protein of av-

erage biologic value 64.5 which is comparable to the protein of wheat, oats, and barley, but less than that of soybean flour. However, when comparing "net protein values," the high protein content of sunflower seed meal (55 per cent) gives it an advantage of 33.7 per cent over soy flour (30.2 per cent), and considerable advantage over wheat (7.6 per cent).

C. R. Grau and H. J. Almquist (*Proc. Soc. Exp. Biol. Med.* **60,** *373 (1945)*) have found sunflower seed meal at a level which supplies 20 per cent protein in the diet of chicks to compare favorably with other recognized complete sources of amino acids. Its high quality is apparent from the growth data on chicks and from the efficiency of food utilization. Even lysine, often deficient in many cereals and oil seed meal proteins, is present in adequate amounts.

These researches indicate that further investigation into the possible utilization of sunflower seed meal as a food for human and animal consumption would be of value. They further emphasize the possibility that continued searches may lead to the discovery of other plant sources of proteins of high biologic value which can be produced at a lower cost than animal proteins. An easily and rapidly produced source of efficient, palatable protein would make an important contribution to the nutrition of the world.

INACTIVATION OF THIAMINE BY CERTAIN FOODSTUFFS

During the course of investigations on the liberation of thiamine and niacin from foodstuffs by enzyme preparations (pig mucosa) K. Bhagvat and P. Devi (*Indian J. Med. Res.* **32,** *131 (1944)*) have observed that certain cereals, legumes, and oil seeds and extracts therefrom rapidly inactivate added thiamine. The properties of the factor indicate that it is not an enzyme and it is therefore not the destroying factor previously found in carp, pike, and certain other raw fish (*Nutrition Reviews* **1,** *403 (1943)*).

When 50 micrograms of thiamine were added to 50 ml. of a buffered (pH 5.6) 10 per cent suspension of rice polishings, ragi, wheat germ, cottonseed, linseed, and certain other seeds, as much as 90 per cent of the thiamine was destroyed overnight. In certain cases after only seven minutes of treatment as much as 75 per cent of added thiamine had disappeared. That the loss of thiamine from solution was not due to adsorption on the solids was shown by the fact that active aqueous extracts free of solids can be prepared. The factor is also extractable in a chloroform water mixture. It is nonextractable by dilute salt solution or acetone. Some activity is obtained in dilute alcohol. The extracts are largely stable to heat including autoclaving. Likewise the thiamine inactivating factor is not affected by acid or alkali at pH 2 or pH 10 for fifteen hours. On dialysis, activity is found on both sides of the membrane; that which is retained by the membrane seems to be heat labile while the inactivating factor of the dialysate is heat stable. The rate of inactivation of thiamine is not influenced by pH over the range 2 to 7. Incubation of yeast or liver with active extracts leads to a complete loss of thiamine from these products.

Although most of the analytical determinations were done by the thiochrome method, biologic assay with rats and pigeons confirmed the loss of the thiamine. Assays using mosquito larvae indicate that active split products of thiamine are, however, present in the incubated solutions. It will be recalled that bisulfite readily inactivates thiamine through fission. Although this is presumably not the factor here, an analogous reaction might be involved. It is also conceivable that materials accelerating the oxidative destruction of thiamine are concerned.

NOTES

Letter to the Editor

Dear Sir:

In (*Nutrition Reviews* **4,** *93* (*1946*)) the statement is made that no tests of biologic activity of riboflavin borate were reported in my paper (*J. Biol. Chem.* **145,** *693* (*1942*)). Evidently, the following statements which appear in my paper were overlooked by the reviewer: "In assays with rats, riboflavin-boric acid complex administered by intramuscular injection three times weekly was as effective in promoting growth as equivalent amounts of riboflavin in water by mouth. The material has also been used successfully by injection to supply the riboflavin requirements of dogs on synthetic rations over periods of several months." Riboflavin-borate also has full biologic activity for Lactobacillus casei. The activity of riboflavin tetrabenzoate was not reported but was found to be nil for L. casei. The latter was not tested on rats.

D. V. Frost,
Nutritional Research Division,
Abbott Laboratories,
North Chicago, Ill.

Diets of Schoolchildren in Industrial Towns in England

As part of an extensive program of nutrition surveys in England during the war, a number of studies on children were made. E. R. Bransby and G. Wagner (*Brit. Med. J.* **2,** *682* (*1945*)) reported analyses of their data collected in two northern English industrial towns during August and September, 1943 and March, 1944. Of over 5000 schoolchildren participating in feeding tests, 426 selected at random provided data on the first survey and 289 of the same individuals were used in the second survey.

The data revealed that the intake of calories and protein, vitamin A, thiamine, and ascorbic acid was adequate but that the calcium intake was 30 to 40 per cent below the standards used. Iron intake was slightly low during the spring survey. When the data were compared on the basis of age by years, the mean caloric and protein intakes were slightly low at 12 to 13 years, iron intake somewhat low above 10 years, and vitamin intake sufficient at all ages. Surprisingly high levels for ascorbic acid were caused by a daily 25 mg. supplement and the chance availability of oranges.

Study of the cost coefficient indicated a general increase in adequacy of intake with increasing expenditure per unit. To some degree this was accounted for by greater use of meats, milk, vegetables, and fruit with less bread as expenditure rose. It is of interest that in homes where the mother worked out either full or part time the children were provided with somewhat poorer meals than when the mother remained at home. A number of interesting conclusions seem to be justified from this survey which includes sociologic as well as dietary findings of note. The average food intake was better than might have been anticipated in industrial towns in England during wartime. The adequacy of the diet rose proportionally with cost, giving further emphasis to the well established relation between economic status and nutritional health. Large families on the average fared a bit worse than smaller ones. Good school feeding was a valuable contribution to the nutrition of the children.

Ascorbic Acid in Pine Needles

Ascorbic acid (vitamin C) is so widely distributed in green leafy plants and is present in such high quantities in a wide variety of native fruits and vegetables, that there are few sections of the world in which there need be a deficiency of this dietary essential, provided full advantage be taken of present and potential scientific knowledge concerning the use of local plant materials. The high ascorbic acid content of rose hips,

evergreen leaves, papayas, currants, gooseberries, and green walnuts has already been mentioned (*Nutrition Reviews* **1,** *246 (1943);* **2,** *287 (1944)*). Scientists of the Soviet Union have reported that pine needles have been found to be a practical source of ascorbic acid, either in the form of simple extracts or as concentrates. A paper from India (N. K. Iyengar, B. C. Bose, and B. Mukerji, *Indian J. Med. Res.* **32,** *165 (1944)*) gives analytical information on the ascorbic acid content of pine needles (Pinus longifolia) available in unlimited quantities in the lower Himalayas. Some data on the stability of the ascorbic acid in pine needle extracts and concentrates are also given.

Fresh pine needles were found to contain from 100 to 175 mg. of ascorbic acid per 100 g. Needles from young trees had the highest content. Green needles cast from the tree and gathered from the ground still contained 25 to 40 mg. of ascorbic acid per 100 g. Simple extracts prepared by grinding in water and filtering through cloth were found to be strongly acid (pH 3.5), a condition favoring the stability of ascorbic acid. The concentrates were prepared by precipitating the gums with methyl alcohol or acetone followed by vacuum distillation of the solvents. Such concentrates (20 mg. ascorbic acid per milliliter) when stored at 4°C. in the absence of air retained more than half their potency for three months. The addition of 60 per cent sugar to the concentrate improved the keeping quality. The extracts were found to be nontoxic to rats and guinea pigs when fed for a period of a month.

The preparation of concentrates of therapeutic value on a large scale in countries like India and China where there is no chemical industry to produce synthetic ascorbic acid would seem to be a valuable expedient.

The more general use of simple extracts of native nontoxic plant materials would undoubtedly improve the ascorbic acid nutrition of many sections of the world.

Thiamine Depletion by Yeast Feeding

H. T. Ness, E. L. Price, and H. T. Parsons (*Science* **103,** *198 (1946)*) have added the feeding of live yeast to the existing technics of dietary restriction and thiaminase destruction of thiamine for the production of thiamine depletion in man. The feeding of live yeast was found to result in urinary thiamine excretion levels characteristic of "considerable to severe deficiency" of thiamine.

Experimentally, 5 college women were placed on a basal diet containing 1.6 mg. of thiamine after a preliminary three day period in which 2 mg. of thiamine daily were administered. Urinary thiamine excretions were determined for three to six days during the basal period, for six to ten days during the additional feeding of 15 or 150 g. of live fresh baker's yeast immediately prior to the meals, and again for a three day basal period after terminating yeast feeding. The feeding of 15 g. of live yeast decreased the urinary thiamine level from 374 to 101 micrograms per day within six days, and 150 g. lowered the excretion to as low as 40 micrograms per day within ten days. The withdrawal of live yeast raised the urinary excretion to levels close to those of the initial basal levels. Boiled yeast did not possess the properties of the live yeast.

It is considered that the effect of live yeast is due to the withholding of the vitamin from absorption. Not only was the effect greater with the larger portion of yeast fed, but an inverse relationship between the urinary and fecal excretion levels during the various experimental periods was noted. Some indication that the body stores of thiamine were decreased during the yeast feeding period was evidenced by the slower return of urinary excretion levels after ten days on the yeast feeding period than after six days. Live yeast feeding may have application in certain experiments in that rapid depletion may be attained without the use of special deficient diets.

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